

Standard Operating Procedure for Derivatization of Macromolecular Crystals with Heavy-atom Reagents

2005 May 12

Introduction

IMCA-CAT authorized staff and users at the IMCA-CAT facility must follow this standard operating procedure for the preparation of heavy-atom derivatives of macromolecular crystals.

Purpose

In order to determine a structure *de novo* using the techniques of macromolecular crystallography, it is necessary to employ either one of two techniques: multiwavelength anomalous diffraction (MAD) or multiple isomorphous replacement (MIR). Some experiments employing MAD and MIR require a method for exposing an already grown macromolecular crystal to a small quantity of a heavy-atom reagent. The purpose of this Standard Operating Procedure is to define a safe and effective procedure for derivatizing macromolecular crystals with heavy-atom reagents.

General Safety Remarks

Most heavy-atom compounds are moderately toxic. The procedure outlined here minimizes the likelihood of exposure to the compound and minimizes generated waste. The IMCA-CAT hood, microscopes, and cold room, all located in the IMCA-CAT Biochemistry Laboratory, are the only locations authorized for use with heavy-atom reagents. All waste generated by this procedure is considered to be hazardous and therefore must be disposed of accordingly. In order to contain the waste, a lab diaper **MUST** be used at all times, except during weighing when the balance may be used without a lab diaper to weigh out solid or liquid heavy-atom reagents. The IMCA-CAT Chemical Safety Coordinator should be consulted for guidance in implementing this procedure, assistance in locating necessary supplies, and instruction in the proper procedure for hazardous waste disposal. Radioactive heavy-atom reagents and compounds are prohibited from use at the IMCA-CAT facilities.

IMCA-CAT staff must be notified in advance of any intent to use heavy-atom reagents.

Please inform your user support staff member or the IMCA-CAT Chemical Safety Coordinator of any intent to use heavy-atom reagents. Notification should be made at least one week in advance of the experiment. Notification should include names of experimenters, dates of experiment, and list of heavy-atom reagents that will be used.

Material Safety Data Sheets must be on file for all heavy-atom compounds to be used.

All heavy-atom reagents transported to or handled at the IMCA-CAT facility must have a Materials Safety Data Sheet (MSDS) on file in the IMCA-CAT MSDS binder. Upon notification of intent to use heavy-atom reagents, the IMCA-CAT Chemical Safety Coordinator will obtain a material safety data sheet (MSDS) for each of the compounds listed for use. This must be done prior to bringing the chemical on site. The user is responsible for reading and understanding the MSDS sections on toxicity and treatment in case of an accident.

Transport and Storage

All heavy-atom compounds are to be stored either in the chemical hood or in a well-ventilated cabinet. Reagents that are to remain at the IMCA-CAT facilities longer than a single visit by a research group will be stored in a limited-access cabinet designated specifically for heavy-atom compounds and reagents. This cabinet is located next to the IMCA-CAT hood. Reagents brought in by users for a single visit to the IMCA-CAT facilities must be transported in a double-container and must be kept in the IMCA-CAT hood. All transport of heavy-atom reagents must be conducted in accordance with ANL Transportation Regulations while on ANL property and with Department of Transportation regulations off of ANL property.

Procedure for Weighing Heavy-Atom Compounds

Wear gloves when handling heavy-atoms and avoid direct contact with the skin. If contact occurs, wash the

contaminated area thoroughly with soap and water. Promptly clean up spills and contact the Chemical Safety Coordinator to determine the appropriate procedure for dealing with a particular compound. To weigh solids, follow this procedure:

1. Tare an appropriately sized container for the amount of solid or solution to be weighed.
2. With gloved hands, open the compound container in a functioning chemical hood and using a clean spatula carefully transfer enough of the solid to the tared vial to meet your requirements.
3. Cap both the bottle and the vial.
4. Wipe the spatula with a Kimwipe.
5. Place the Kimwipe into the hazardous waste disposal container and the spatula in the wash bucket.
5. Reweigh the vial to determine the actual amount of solid present and add water or solvent to dissolve the solid. Store the stock heavy-atom solution in the hood, either in the user-supplied double container or the IMCA-CAT storage cabinet.
6. Dispose gloves into hazardous waste bag.

Procedure for soaking heavy-atom reagents into macromolecular crystals

Soaking heavy-atom reagents into macromolecular crystals is a standard technique for obtaining heavy-atom derivatives of macromolecular crystals. (See reference below.) Typically the preformed crystals are placed in artificial mother liquors containing various concentrations of heavy-atom compounds. In order to keep the volumes of heavy-atom reagent that will be added to the crystal mother liquor small (less than 10% of original droplet volume), relatively high stock concentrations (20 to 50mM or higher) of the heavy atom compounds need to be made. With the crystal sitting in a small drop (~10 μ l) of the artificial mother liquor, a small amount (~0.2 μ l) of the heavy-atom solution is added. The crystal is observed under a microscope for a few minutes to detect signs of disruption to the crystal lattice, such as crystal cracking, loss of birefringence or melting. If there are no signs of disruption to the crystal lattice, then additional aliquots of heavy-atom solution may be added until the final heavy-atom concentration is between typically 2 to 5mM. The crystal may be left undisturbed for a period of time (minutes to days). The crystal may change color, depending upon the compound used. In order to reduce non-specific binding, the crystal may be back-soaked for a period of time (minutes to hours) in heavy-atom-free mother liquor.

Exclusions

Radioactive Samples:

Radioactive substances and radioactive heavy-atom reagents, including ^{32}P , ^{35}S , and ^{125}I , are not permitted at the IMCA-CAT facilities. Reactions involving compounds labeled with these radioactive nuclides should be performed in the users' home laboratories. Crystals already derivatized with these substances are permitted at IMCA-CAT because the level of radioactivity actually contained in the crystal is typically below the levels defined by the Department of Energy set forth in Chapter 5.2, Table 02-3 of the Argonne Environmental, Safety, and Health Manual. IMCA-CAT staff must be informed in advance if such crystals are to be transported to the IMCA-CAT facilities.

Volatile Heavy-Atoms:

Extremely volatile heavy-atom compounds, including dimethyl mercury, tetramethyl lead, and tetraethyl lead, are not permitted at the IMCA-CAT facilities. Reactions involving compounds labeled with these volatile heavy atom compounds should be performed in the users' home laboratories. Crystals already derivatized with these compounds are permitted provided that the total concentration of toxic substance present falls below the maximum allowable levels recommended by APS ES&H. IMCA-CAT staff must be informed in advance if such crystals are to be transported to the IMCA-CAT facilities.

Pathogens:

All pathogenic organisms are prohibited from the IMCA-CAT facilities.

References

Rould, M.A. "Screening for Heavy-Atom Derivatives and Obtaining Accurate Isomorphous Differences" *Methods in Enzymology* Vol 276 Part A: 461-472 (1997) or Blundell, T.L.; Johnson, L.N. "Preparation of Heavy Atom Derivatives" *Protein Crystallography* Chapter 8: 183-239 (1976) and references contained therein for a more thorough discussion of the preparation of heavy-atom derivative crystals.)